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(54) Title: NEUROPEPTIDE Y ANTAGONISTS AND AGONISTS

(57) Abstract

The invention discloses analogs which behave as NPY antagonists and agonists; and methods of their use for controlling a biological activity such as appetite and cardiovascular function.

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NEUROPEPTIDE Y ANTAGONISTS AND AGONISTS

Background of the Invention

This invention relates to peptide derivatives which are antagonists or agonists of neuropeptide Y.

Neuropeptide Y (NPY), is a 36-residue peptide characterized by a tyrosine (Y) residue at its N-terminus and a tyrosine amide residue at its C-terminus. The peptide was isolated from porcine brain (Tatemoto *Proc. Natl. Acad. Sci.* U.S.A. 79:5485-5489, 1982) and is considered to be a neurotransmitter or neuromodulator widely distributed in the central and peripheral nervous systems (Allen et al., *Neurochem. Int.* 8:1-8, 1986). It is the most abundant peptide present in the mammalian brain and heart (Edvinsson et al., *Trends Pharmol. Sci.* 8:231-235, 1987; Gu et al., *Histochem. Cytochem.* 32:467-472, 1984), and is among the most potent vasoconstrictor peptides isolated to date (Lundberg et al., *Acta Physiol. Scand.* 121:325-332, 1984). However, NPY elicits several physiological responses by activating specific pre- and post-synaptic receptors. Centrally, NPY is thought to be involved in the regulation of food intake, memory processing and circadian rhythm (Sheikh et al., *FEBS Lett.* 245: 209-214, 1989). In the periphery, NPY seems to function as a transmitter in sympathetic nerves where it interacts with norepinephrine mainly in the regulation of vasculartone (Sheikh et al. *FEBS Lett.* 245:209-214, 1989).

Different structure-activity relationships for NPY analogs in various model systems have indicated that multiple NPY receptor subtypes exist (Michel, *Tips* 12:389-394, 1991). Wahlestedt and coworkers (*Regul. Pept.* 13:307-318, 1986) first suggested the existence of two distinct subtypes of NPY receptors. Post-synaptic (Y1-type) effects could be obtained with the complete NPY

molecule, while pre-synaptic (Y2-type) effects were found elicited by long C-terminal fragments, as well as with the entire NPY molecule. Thus, both Y1 and Y2 receptors exhibit nearly equal affinity to NPY and its homologous peptide, peptide YY, but only the Y2 receptors could bind to shorter carboxyl-terminal fragments including NPY(13-36) as described by Sheikh et al. (*FEBS Lett.* 245:209-214, 1989). However, since NPY receptors in rat cardiac ventricular membranes discriminate between NPY and peptide YY but bind NPY(13-36), it was suggested that this system be classified as a subtype of Y2 or a new class (designated Y3) of receptors as discussed below (Balasubramaniam et al. *Peptides* 11:545-550, 1990).

NPY is also present in high concentrations in a distinct population of nerve fibers innervating the heart and blood vessels (Wharton et al., *Ann. N.Y. Acad. Sci.* 611:133-144, 1990). NPY is now regarded as the predominant peptide present in the cardiovascular system of mammals. This observation has led to numerous studies of the cardiovascular properties of NPY. For example, several investigations have reported that NPY is a potent vasopressor peptide and that it inhibits the coronary blood flow and contractility in isolated perfused hearts (e.g., see Balasubramaniam et al., *Regul. Pept.* 21:289-299, 1988; Allen et al. *Regul. Pept.* 6:247-253, 1983; Rioux et al. *Peptides* 7:27-31, 1986). Furthermore, NPY is also capable of (1) inhibiting the contractile force of isolated cardiac muscles (Balasubramaniam et al. *supra*) and myocytes (Piper et al. *Nuanyn-Scniedberg's Arch. Pharmol.* 340: 333-337, 1989) and (2) the adenylate cyclase activity and cAMP production by cardiac muscles (Kassis et al., J. Biol. Chem. 262: 3429-3431, 1987) and myocytes (Kassis et al.*supra*; Millar et al. *Nuanyn-Scniedberg's Arch. Pharmol.* 338:426-429, 1989), respectively. Specific receptors of NPY in rat cardiac

ventricular membranes have been characterized and reported to be more selective to NPY than previously characterized NPY receptors as discussed above (Balasubramaniam et al. *Peptides* 11:545-550, 1990). The presence of specific receptors of NPY in rat cardiac membranes, the Y-3 receptor, was also reported by visualization with N^{α} biotinyl-NPY analogs (Balasubramaniam et al. *Peptides* 11: 1151-1155, 1990).

The following table (the abbreviations used are commonly known in the art and are described *infra*) shows the amino acid homology between NPY and PYY:

	5 10 15 20 25 30 35
Human NPY	YPSKPDNPGEDAPAEDMARYYSALRHYINLITRQRY
Rat NPY	YPSKPDNPGEDAPAEDMARYYSALRHYINLITRQRY
Rabbit NPY	YPSKPDNPGEDAPAEDMARYYSALRHYINLITRQRY
Guinea pig NPY	YPSKPDNPGEDAPAEDMARYYSALRHYINLITRQRY
Porcine NPY	YPSKPDNPGEDAPAEDLARYYSALRHYINLITRQRY
Human PYY	YPIKPEAPGEDASPEELNRYYASLRHYLNLVTRQRY
Porcine PYY	--A----------S----------
Rat PYY	--A----------S----------

NPY has been implicated in the pathophysiology of a number of diseases including, without limitation, obesity, hypertension and chronic heart failure (CHF) because: (1) hypothalamic NPY levels are elevated in obese rats and decreased in cancer anorectic rats; (2) plasma NPY levels are elevated in CHF and hypertensive patients; (3) negative cardiac inotropic and chronotropic actions; and (4) inhibition of libido and circadian rhythm. Thus, since NPY has been shown to be important for regulating a plurality of physiological events we have set out to design a series of receptor-specific analogs that selectively modulate a variety of biological activities, e.g., appetite and blood pressure activities.